Letters to the Editor

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Etruscan Artifacts

To the Editor:

Recently, Vernesi et al. (2004) attempted to determine the variation in the first hypervariable segment (HVS-I) of mtDNA extracted from a number of Etruscan teeth. However, the rather unusual variation reported calls authenticity of the ancient mtDNA into question.

Multiple occurrences of the same tandem mutation in lineages from disjoint haplogroups distinctly signal artifacts (Bandelt et al. 2002). For instance, the "Etruscan" data harbor the transition pair 16193-16219 thrice, on quite different HVS-I backgrounds, even separated by a restriction site—once jointly with +14766 *Mse*I and twice with −14766 *Mse*I. The transition 16219, however, is quite a rare mutation, which is confined essentially to haplogroup U6ab (Maca-Meyer et al. 2003) and to a specific subclade of haplogroup H that bears the two characteristic mutations 16482 and 239. In the latter case, one indeed finds the motif 16193-16219-16362 in the worldwide database—for example, in Great Britain (Richards et al. 2000), in Germany (Pfeiffer et al. 1999), and in the United States (as recorded in the SWGDAM database [Monson et al. 2002]); however, in the study by Vernesi et al. (2004), the motif 16193-16219 occurs without 16362.

The mutation 16069, which almost always signals haplogroup J, is not seen in the "Etruscan" mtDNA data in combination with the 16126 transition or with +14766 *Mse*I but is recorded twice with −14766 *Mse*I. Could this surprising feature be explained by recurrent mutations at 16069? Hardly—the 16069 transition is not among the "speedy" transitions reported by Bandelt et al. (2002). The mtDNA lineages with motif 16126 (outside haplogroup H), 16126-16193, and 16126-16193-16278 are normally seen together with 16069 in the West Eurasian mtDNA pool (except in the evidently flawed HVS-I data of Fraumene et al. [2003]). Therefore, we have to expect at least four independent mutations at site 16069 in the "Etruscan" mtDNA data—if we do not want to invoke de novo mutations at 16193 and 16278 (and this without a single trace of any familiar haplogroup J lineage in this data set). The 16069 transition on non-J lineages has been observed earlier in the "Ladins" (Stenico et al. 1996), where it occurs three times (in tandem with the 16085 transition). The variation at 16069 in these data sets thus seems abnormal in comparison with the worldwide mtDNA database.

Vernesi et al. (2004) rejected the idea that postmortem damage could have been responsible for the assumed back mutations at 16069 (or 16294), with reference to the study of Gilbert et al. (2003), inasmuch as no such case was observed there. This argument is, however, invalid, since the regular haplogroup J (or T, respectively) nucleotide T at 16069 (or 16294, respectively) is less frequent by 1 order of magnitude than the corresponding majority nucleotide C (of the Cambridge reference sequence), so that no significant inference could be drawn from Gilbert et al. (2003) as it was for asymmetric transition probabilities (C→T vs. T→C) at these sites.

There are numerous technical flaws in figure 2 and table 1 of Vernesi et al. (2004)—in addition to the potential sequencing problems—thus additionally undermining confidence in their ancient mtDNA data. Haplotype 6AM is not well defined, because the corresponding HVS-I sequences (from Adria and Magliano/Marsiliana [Vernesi et al. 2004]) are observed with both +/−14766 *Mse*I, according to their table 1. Haplotype 13C is misplaced in figure 2, since it bears +14766 *Mse*I. This figure certainly does not present a reduced median network (as claimed), because the Adria 6AM haplotype should be a neighbor of haplotype 14CMT. It is not at all clear how this network was actually constructed, because, in the article, the reference is given to the median-joining algorithm (Bandelt et al. 1999), which is fundamentally different from the reduced-median algorithm (Bandelt et al. 2000). The median-joining algorithm, however, would instead reconstruct a triangle for site 16095, in this case. In the data set, the 16223 transition relative to rCRS is observed twice in connection with −14766 *Mse*I, which otherwise should be more the exception than the rule, since rCRS is the ancestral HVS-I motif of haplogroup HV (as well as of the superhaplogroup R). On the other hand, this restriction site has not been determined for haplotype 9A, yet it is reconstructed in the network, not most parsimoniously, as +14766 *Mse*I. Finally, the node sizes in figure 2 do not always correspond to the frequencies recorded in table 1 of the article.

The assertion that "all the strictest criteria for the val-

idation of ancient DNA sequences have been followed" (Vernesi et al. 2004 [p. 703]) is not quite correct, since one of the most important criteria of Cooper and Poinar (2000)—that is, that of independent replication in another lab—has not been followed for 25 of 28 of the reported HVS-I sequences or for any of the RFLP tests. Moreover, the 20 excluded sequences were not displayed. The claim that the "Etruscan" sequences "all belong to lineages that are still present in Europe" (Vernesi et al. 2004 [p. 702]) is not justified, in view of the unusual mutational pattern, especially as the basal haplogroup status (U, JT, pre-HV, N1, W, X, or other) was not determined in half of the data set. Under these circumstances, it is unclear to what extent the "Etruscan" data represent severely damaged or partly contaminated mtDNA sequences; therefore, any comparison with modern population data must be considered quite hazardous.

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On the Etruscan Mitochondrial DNA Contribution to Modern Humans

To the Editor:

The growing number of ancient human mtDNA samples sequenced in recent years has given rise to the problem of correspondence between distributions of mutations in ancient and modern mtDNA sequences. It has been suggested that mtDNA nucleotide sequences obtained from human remains may include some artifacts, for multiple reasons, such as contamination with modern DNA; artifacts induced by cytosine deamination during multiple amplification of ancient DNA via PCR; and postmortem damage in DNA, occurring as hydrolytic deamination and depurination, double-strand breaks, and oxidative nucleotide modification (Hofreiter et al. 2001*a*). Therefore, to determine the nature of the DNA sequences amplified, each amplified product should be cloned, and the obtained clones should be sequenced (Pääbo 1989; Handt et al. 1996). The consensus sequence from each sample is determined from the sequences shared between all clones, and intraclone nucleotide differences represent the postmortem data set (Gilbert et al. 2003). Therefore, cloned sequences of ancient DNA samples may show a pattern of a shared consensus (haplotype), with many singleton substitutions corresponding to postmortem DNA changes. It has been suggested that the consensus sequence should be part of the original sequence (Hofreiter et al. 2001*b*).

In this study, we reanalyzed nucleotide sequences of the mtDNA HVS-I region in 575 clones derived from bone samples of 28 Etruscans (7th–3rd centuries B.C.),